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Title: The use of a plant enzyme provides a fast, sensitive and reproducible LC-MS/MS method for folate analyses in food

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Folate analyses in food are one of the most challenging due to the structural variability, low stability and low presence of folates, but also because of the complexity of food matrices. The use of protease, amylase and deconjugase of animal origin, known as a tri-enzyme extraction, has been considered as a necessity in order to provide the complete determination of folates in food. However, there are discrepancies in the data for folate content between methods due to the lack of a proper deconjugase enzyme which would enable fast and reproducible folate deglutamylation.

The newly developed method included single-enzymatic extraction step in which an enzyme of a plant origin (Arabidopsis thaliana) has been used. Seven folate vitamers (tetrahydrofolate, 5,10-methenyl-tetrahydrofolate, 10-formyl-folic acid, 5-formyl-tetrahydrofolate, folic acid, 5-methyl-tetrahydrofolate and 10-methyl-folic acid) were quantified using the 13C5-labelled internal standards by LC-MS/MS method. Deconjugase of a plant origin provided sufficient folate deglutamylation within 1 hour, showing the activity >95%. Consequently, it enabled performance of the whole analysis within 1 working day, omitting commonly used overnight incubation. The method was validated to assess precision (±10% RSD) and trueness (80-120%). Additionally, 89 samples of dairy products, fruit, vegetables, legumes, cereals, offal and meat were analyzed with this new method, another LC-MS/MS method using rat serum deconjugase, and the traditional microbiological assay.

Furthermore, the mobile phases, column and the MS-ion source used in this method, may be used for determination of vitamins D and K, which provides an easy shift in the analyses of these vitamins in the lab.